

Selection for Divergent Ferulate Cross-Linking and Klason Lignin Concentrations in Smooth Bromegrass

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Introduction

Research by the USDFRC Cell Wall Group has demonstrated that cell walls of grasses have extensive cross-linking of arabinoxylans to lignin by ferulic acid bridges. Using primary cell walls from corn tissue cultures that have been induced to lignify, the degree of ferulate cross-linking is strongly correlated with cell-wall degradability. However, we have had less success showing similar correlations of cross-linking with degradability for intact plants. There is generally a positive correlation between Klason lignin concentration and ferulate cross-linking in grasses. This raises the question of whether cross-linking has a direct effect on cell-wall degradability or if it is merely a reflection of the correlation between cross-linking and lignin. We undertook a selection study in smooth bromegrass to identify genotypes that exhibit repeatable differences in ferulate cross-linking and Klason lignin concentration to more directly determine the independent effects of these two components of grass cell-wall structure on cell-wall degradability.

Materials and Methods

Leaf tissue at the vegetative stage of maturity was collected in 1992 from 300 individual plants taken from each of four smooth bromegrass populations: the cultivars Alpha and Lincoln, and the two synthetic populations WB19e and WB88S derived from elite breeding lines and wild germplasm collected in Russia, respectively. All samples were oven dried, ground, and scanned by near-infrared spectroscopy (NIRS). Eighty samples were analyzed by wet-chemistry for neutral detergent fiber (NDF), ferulate ethers (the only form of cross-linking that can be quantified), and Klason lignin by standard methods. These data were used to generate NIRS calibration equations and then predict composition of all 1200 leaf samples. Ferulate ethers and Klason lignin concentrations were examined on a NDF basis. Twenty plants (five per population) were selected for each of four selection groups; low ferulate ethers: low lignin, high ferulate ethers: low lignin, low ferulate ethers: high lignin, and

high ferulate ethers: high lignin. In 1993 the 80 selected plants were re-sampled as done in 1992. All samples were analyzed for ferulate ethers and Klason lignin by wet-chemistry. Based on the means over the two sampling years, 32 plants (two plants per population per selection group) were selected for further evaluation. These 32 plants were clonally propagated and transplanted into a replicated randomized complete block design field trial at Arlington, WI in 1994. Leaf samples were collected from two harvests at the vegetative maturity stage in both 1995 and 1996. All samples were analyzed by wet-chemistry for NDF, etherified ferulic acid, Klason lignin, and 24- and 96-h in vitro NDF degradability.

Results and Discussion

Both ferulate ethers and Klason lignin concentration showed considerable phenotypic variation in all four populations. Based on the two phases of selection (1992 and 1993), it was apparent that both traits were subject to substantial genotype x environment interactions. The results for the 32 selected clones evaluated in 1995 and 1996 are shown in Fig. 1. Selection was successful for ferulate ethers in all populations except WB88S, creating repeatable divergence of 11.2 to 12.5%. Selection was unsuccessful for Klason lignin concentration, most likely due to the genotype x environment interactions. While we were not successful in creating the four targeted divergent phenotypes, the resulting clones showed significant variation for both traits and these two traits were independent ($r = 0.08$; $P > 0.05$) among the selected clones. Both lignin concentration and cross-linking had significant negative effects on 96-h in vitro NDF degradability across three different statistical estimation methods, but little effect on 24-h NDF degradability was observed. For the most divergent and independent clonal comparisons of cross-linking and lignin impact on NDF degradability, 75% of the contrasts showed a significant negative effect of high cross-linking and lignin concentration on degradability (Table 1).

Conclusions

Selection for divergent ferulate cross-linking in smooth bromegrass was successful. Both Klason lignin and ferulate ethers had negative impacts on in vitro NDF degradability, and these effects were of similar magnitude and independent. Our results indicate that recurrent selection for reduced ferulate cross-linking in smooth bromegrass should be a successful strategy for

improving forage quality; however, the large genotype x environment interaction will make progress slow. The most divergent clones identified to date provide a valuable resource for conducting animal feeding trials to evaluate the impact of ferulate cross-linking and lignin concentration on animal performance. These genetic materials offer the opportunity to test the impact of these traits in vivo, which has not been possible previously.

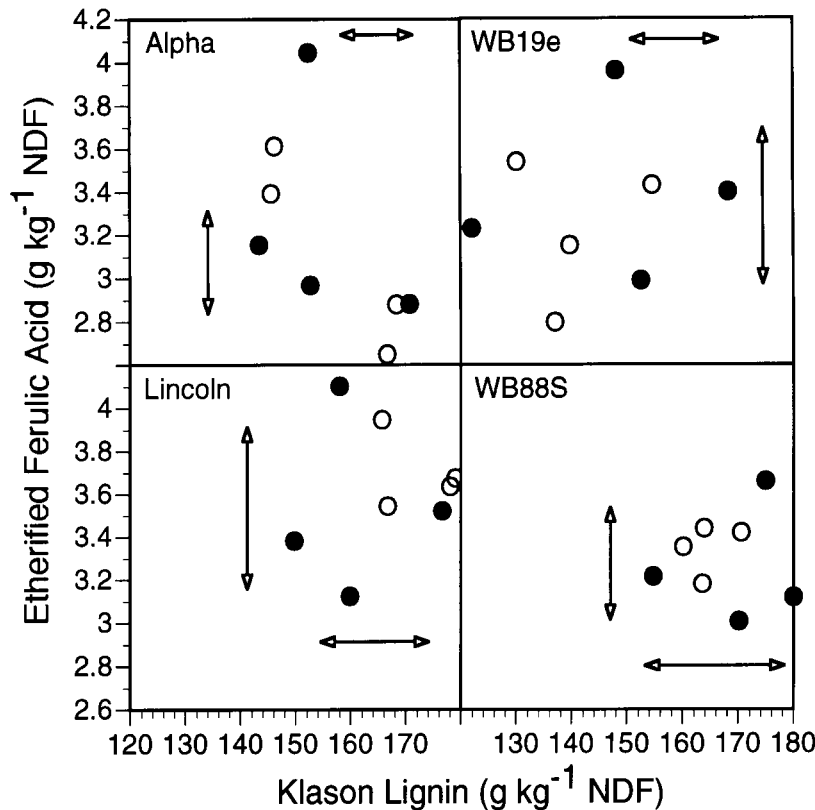


Figure 1. Scatter plots of etherified ferulic acid vs. Klason lignin concentration for clone means of four smooth bromegrass populations. Double-headed arrows represent LSD values for clone means within populations ($P < 0.01$). Closed symbols represent the most desirable clones within each population for testing the independent effects of lignin concentration and cross-linking on cell-wall degradability.

Table 1. Mean differences from high vs. low clonal comparisons for etherified ferulic acid, Klason lignin, and 24- and 96-h in vitro NDF degradability in smooth bromegrass.

Population	Clonal Comparison	Impact of Divergence			
		Concentration		In Vitro NDF Degradability	
		Ferulate Ether	Klason Lignin	24-h	96-h
		----- g kg ⁻¹ NDF -----		----- g kg ⁻¹ -----	
Ferulate Contrast					
Alpha	5 - 4	1.07**	0.3	-33	-51**
WB19e	1 - 4	0.97**	-4.5	-50*	-22*
Lincoln	3 - 4	0.98**	-1.9	-23	-11
WB88S	8 - 2	0.65**	4.8	-10	-30*
Klason Lignin Contrast					
Alpha	6 - 1	-0.28	27.3**	-8	6
WB19e	3 - 7	0.17	46.3**	-26	-28**
Lincoln	1 - 6	0.29	29.3**	-8	-25*
WB88S	4 - 7	-0.10	25.5**	-19	-30*

***Effect is significantly different from zero ($P < 0.05$ and 0.01 , respectively).